PATENT COOPERATION TREATY

CC: PRINCETON

BTB

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

To:
WYETH
Patent Law Department
Attn. Calnan, William H.
Five Giralda Farms
Madison, New Jersey 07940
UNITED STATES OF AMERICA

RECEIVED
NOV 15 2004
Bill T. Brazil

Applicant's or agent's file reference: AM100485	Date of mailing (day/month/year): 04/11/2004
International application No.: PCT/US2004/007673	FOR FURTHER ACTION: See paragraphs 1 and 4 below
Applicant: WYETH HOLDINGS CORPORATION	International filing date (day/month/year): 11/03/2004

1. [x] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until **30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority: European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer: Michela Digiusto

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By

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims,description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Annexes B1 and B2).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, Volume I/A, paragraph 296).

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

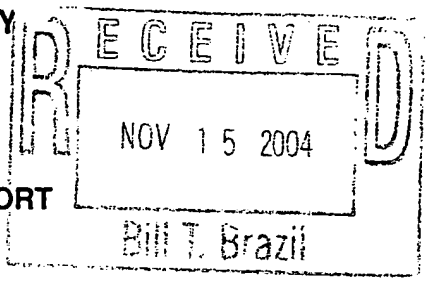
The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

PATENT COOPERATION TREATY

PCT



INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
AM100485		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2004/007673	11/03/2004	17/03/2003
Applicant		
WYETH HOLDINGS CORPORATION		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 9 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      [ ] The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box II).

3. [X] **Unity of invention is lacking** (see Box III).

4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regards to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant.
      [ ] as selected by this Authority, because the applicant failed to suggest a figure.
      [ ] as selected by this Authority, because this figure better characterizes the invention.
   b. [X] none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/007673

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material

   - [ ] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material

   - [X] in written format
   - [X] in computer readable form

   c. time of filing/furnishing

   - [X] contained in the international application as filed
   - [X] filed together with the international application in computer readable form
   - [ ] furnished subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

ternational Application No
PCT/US2004/007673

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K19/00 A61P31/00
//C07K14/245,C07K14/28,C07K14/235

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, PAJ, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAJISHENGALLIS G ET AL: "MUCOSAL IMMUNIZATION WITH A BACTERIAL PROTEIN ANTIGEN GENETICALLY COUPLED TO CHOLERA TOXIN A2/B SUBUNITS" JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 154, no. 9, 1 May 1995 (1995-05-01), pages 4322-4332, XP000645280 ISSN: 0022-1767 abstract; figure 5	1-3, 6-15, 29-34, 52-54, 57, 59-66, 80,82-85
Y		4,5, 16-28, 55,56, 58,67-79

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 2004	04. 11. 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,	Wagner R

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98/42375 A (CHIRON CORP) 1 October 1998 (1998-10-01) cited in the application the whole document	4,5, 16-28, 36-43, 55,56, 58, 67-79, 87-94
Y	WO 97/02348 A (GIANNELLI VALENTINA ; PIZZA MARIAGRAZIA (IT); BIOCINE SPA (IT); RAPPUO) 23 January 1997 (1997-01-23) cited in the application the whole document	4,5, 16-28, 55,56, 58,67-79
Y	WO 93/13202 A (SCLAVO BIOCINE SPA) 8 July 1993 (1993-07-08) cited in the application the whole document	4,5, 16-28, 55,56, 58,67-79
Y	WO 00/18434 A (JOBLING MICHAEL G ; GREEN BRUCE A (US); PEEK JOEL A (US); US HEALTH (U) 6 April 2000 (2000-04-06) the whole document	4,5, 16-28, 55,56, 58,67-79
X	MARTIN M ET AL: "Recombinant antigen-enterotoxin A2/B chimeric mucosal immunogens differentially enhance antibody responses and B7-dependent costimulation of CD4+ T cells" INFECTION AND IMMUNITY, AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, US, vol. 69, no. 1, January 2001 (2001-01), pages 252-261, XP002231728 ISSN: 0019-9567 abstract	1,12,52, 63
X	JOBLING MICHAEL G ET AL: "Fusion proteins containing the A2 domain of cholera toxin assemble with B polypeptides of cholera toxin to form immunoreactive and functional holotoxin-like chimeras" INFECTION AND IMMUNITY, vol. 60, no. 11, 1992, pages 4915-4924, XP002292263 ISSN: 0019-9567 abstract	1,12,52, 63
A	US 6 395 964 B1 (ARNTZEN CHARLES J ET AL) 28 May 2002 (2002-05-28) column 17	1-34, 52-85

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93/07178 A (PASTEUR MERIEUX SERUMS VACC) 15 April 1993 (1993-04-15) page 10 - page 12; claim 10	44, 95-101
X	WO 01/74383 A (UAB RESEARCH FOUNDATION) 11 October 2001 (2001-10-11)	35,86
Y	figure 1	36-43, 87-94
Y	GIANNELLI V ET AL: "Protease susceptibility and toxicity of heat-labile enterotoxins with a mutation in the active site or in the protease-sensitive loop." INFECTION AND IMMUNITY. JAN 1997, vol. 65, no. 1, January 1997 (1997-01), pages 331-334, XP002301115 ISSN: 0019-9567 page 331	36-43, 87-94
X	SCHNEERSON R ET AL: "SYNTHESIS OF A CONJUGATE VACCINE COMPOSED OF PNEUMOCOCCUS TYPE 14 CAPSULAR POLYSACCHARIDE BOUND TO PERTUSSIS TOXIN" INFECTION AND IMMUNITY, AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, US, vol. 60, no. 9, 1 September 1992 (1992-09-01), pages 3528-3532, XP000371779 ISSN: 0019-9567 abstract	44,95
X	WO 02/47727 A (WILSON ANDREW DOUGLAS ; ONG KONG WEE (GB); UNIV BRISTOL (GB); MORGAN A) 20 June 2002 (2002-06-20) claim 14	35,86
X	CARBONETTI NICHOLAS H ET AL: "Stimulation of HIV gp120-specific cytolytic T lymphocyte responses in vitro and in vivo using a detoxified pertussis toxin vector" AIDS RESEARCH AND HUMAN RETROVIRUSES, vol. 17, no. 9, 10 June 2001 (2001-06-10), pages 819-827, XP002301116 ISSN: 0889-2229 page 820	44-51, 95-101

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

International Application No
PCT/US2004/007673

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9842375 A	01-10-1998	AU 741902 B2	13-12-2001
		AU 6571398 A	20-10-1998
		CA 2284541 A1	01-10-1998
		EP 0971738 A1	19-01-2000
		JP 2001517233 T	02-10-2001
		NZ 500159 A	24-11-2000
		WO 9842375 A1	01-10-1998
WO 9702348 A	23-01-1997	AU 6238896 A	05-02-1997
		EP 0835314 A1	15-04-1998
		WO 9702348 A1	23-01-1997
		US 2004137017 A1	15-07-2004
		US 2002044939 A1	18-04-2002
WO 9313202 A	08-07-1993	IT 1253009 B	10-07-1995
		AT 177145 T	15-03-1999
		AU 3347693 A	28-07-1993
		CA 2127091 A1	08-07-1993
		DE 69228563 D1	08-04-1999
		DE 69228563 T2	29-07-1999
		DK 620850 T3	27-09-1999
		WO 9313202 A1	08-07-1993
		EP 0620850 A1	26-10-1994
		EP 0869181 A1	07-10-1998
		ES 2127808 T3	01-05-1999
		GR 3029556 T3	30-06-1999
		JP 7506240 T	13-07-1995
		JP 3394774 B2	07-04-2003
		JP 2003000287 A	07-01-2003
		MX 9207685 A1	31-05-1994
		SG 48217 A1	17-04-1998
		SG 93200 A1	17-12-2002
		US 2004137017 A1	15-07-2004
		US 2002044939 A1	18-04-2002
		US 6149919 A	21-11-2000
WO 0018434 A	06-04-2000	AU 770333 B2	19-02-2004
		AU 6403999 A	17-04-2000
		BR 9914160 A	26-06-2001
		CA 2344740 A1	06-04-2000
		CN 1320043 T	31-10-2001
		EP 1117435 A1	25-07-2001
		JP 2002525093 T	13-08-2002
		WO 0018434 A1	06-04-2000
US 6395964 B1	28-05-2002	AU 691707 B2	21-05-1998
		AU 4194096 A	15-05-1996
		EP 0793717 A1	10-09-1997
		JP 10507916 T	04-08-1998
		NZ 297142 A	28-09-2001
WO 9307178 A	15-04-1993	FR 2682388 A1	16-04-1993
		AT 217015 T	15-05-2002
		AU 661071 B2	13-07-1995
		AU 2946992 A	03-05-1993
		CA 2098105 A1	10-04-1993
		DE 69232585 D1	06-06-2002
		DE 69232585 T2	05-12-2002

INTERNATIONAL SEARCH REPORT

...formation on patent family members

International Application No
PCT/US2004/007673

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9307178 A		DK	562107 T3	19-08-2002
		EP	0562107 A1	29-09-1993
		ES	2174839 T3	16-11-2002
		FI	932626 A	09-06-1993
		WO	9307178 A1	15-04-1993
		HU	70298 A2	28-09-1995
		JP	6506233 T	14-07-1994
		KR	249709 B1	15-03-2000
		NO	932102 A	05-08-1993
		US	6007818 A	28-12-1999
		US	6045805 A	04-04-2000
WO 0174383 A	11-10-2001	AU	5521701 A	15-10-2001
		WO	0174383 A1	11-10-2001
		US	2002004238 A1	10-01-2002
WO 0247727 A	20-06-2002	AU	2215302 A	24-06-2002
		CA	2434915 A1	20-06-2002
		EP	1351708 A1	15-10-2003
		WO	0247727 A1	20-06-2002
		US	2004067240 A1	08-04-2004

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/007673

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-11, 12-34, 52-62, 63-85

Claims 1-11, 12-34, 52-62, 63-85 which are directed to an antigen covalently associated to a mutated cholera holotoxin (CT)

---

2. claims: 35-43, 86-94

Claims 35-43, 86-94, which are directed to an antigen covalently associated to an Escherichia Coli heat labile toxin (LT).

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3. claims: 44-51,95-101

Claims 44-51, 95-101, which are directed to an antigen covalently associated with a pertussis toxin (PT).

PATENT COOPERATION T .ATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/007673	11.03.2004	17.03.2003

International Patent Classification (IPC) or both national classification and IPC
C07K19/00, A61P31/00

Applicant
WYETH HOLDINGS CORPORATION

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d

Authorized Officer

Wagner, R

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/007673

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 52-95 (IA)

because:

☒ the said international application, or the said claims Nos. 52-95(IA) relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4,5,16-28,36-43,47,48,49,50,55,56,58,67-79,81,87-94
	No:	Claims	1-3,6-15,29-34,35,44-46,51,52-54,57,59-62,63-66,80,82-85,86,95,96,97-101
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-101
Industrial applicability (IA)	Yes:	Claims	1-51,96-101
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 52-95 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item IV

Lack of unity of invention

The present set of claims comprises 7 independent claims which are directed to compositions or methods of treatment comprising ADP-ribosylating toxins, which are mutated (claims 1, 12, 52, 63) or not (claims 35, 86, 95) and which are all covalently associated with an antigen. The covalently linked toxin increases the immunogenicity of the antigen.

The general concept which can be identified among those independent claims is the fact that the covalent linkage of an ADP-ribosylating toxin (mutated or not) to an antigen, increases the immunogenicity of said antigen.
This link cannot be considered as a single inventive concept in the sense of Rule 13.2 PCT for the following reasons:
D1 (Hajishengallis et al., The Journal of Immunology, 1995, 154, 4322-4332) discloses the linkage of the antigen -Saliva Binding Region of the streptococcal protein adhesin AgI/II- to Cholera Toxin A2/B, which is an ADP-ribosylating toxin in which a deletion (i.e. a mutation by deletion) of the fragment A1 of the A subunit was carried out. D1 discloses that the entire antigen protein AgI/II induces S-IgA only when the antigen in conjugated to CTB, which implies that the protein alone does not induce S-IgA. The Saliva Binding Region of the streptococcal adhesin is however immunogenic (see figure 5, b) when conjugated to Cholera Toxin A2/B. Therefore D1 anticipates the concept of a mutated ADP-ribosylating toxin linked to an antigen.

D2 (US6395964) discloses that antigens can be fused to LT or CT subunits and that the subunits are able to form holotoxins (col17, line 15-39). Thus D2 anticipates the

concept that the antigen is fused to a non-mutated ADP-ribosylating toxin.

The requisite unity of invention (Rule 13.1 PCT) does not no longer exist inasmuch as a technical relationship involving one or more of the same or corresponding special technical features in the sense of Rule 13.2 PCT does not exist between the subject-matter of the following groups of claims:

Invention A: Claims 1-11, 12-34, 52-62, 63-85 which are directed to an antigen covalently associated to a mutated cholera holotoxin (CT)

Invention B: Claims 35-43, 86-94, which are directed to an antigen covalently associated to an Escherichia Coli heat labile toxin (LT).

Invention C: Claims 44-51, 95-101, which are directed to an antigen covalently associated with a pertussis toxin (PT).

The features which link the subject-matter of the claims within the respective groups are the specific structural feature of respectively CT, LT and PT

In conclusion, the above groups of claims are not linked by common or corresponding special technical features and define 3 different inventions not linked by a single general inventive concept.

The application, hence does not meet the requirements of unity of invention as defined in Rules 13.1 and 13.2 PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:
D1: Hajishengallis et al., The Journal of Immunology, 1995, 154, 4322-4332.
D2: US 6395964

D3: WO98/42375
D4: WO97/02348
D5: WO93/13202
D6: WO00/18434
D7: Martin et al., Infection and Immunity, vol. 69, no.1, 2001, pp. 252-261.
D8: Jobling and Holmes, Infection and Immunity vol. 60, no. 11, 1992, pp. 4915-4924
D9: WO 02/47727
D10: WO01/74383
D11: Gianelli et al., Infection and Immunity, Jan. 1997, p. 331-334.
D12: Schneerson et al., Infection and Immunity, Sept. 1992, p3528-3532.
D13: WO93/07178
D14: Carbonetti et al, Aids Research and Human Retroviruses, Vol. 17. no. 9, 2001, pp. 819-827.

Invention A: Claims 1-11, 12-34, 52-62, 63-85 which are directed to an antigen covalently associated to a mutated cholera holotoxin (CT)

1. Independent claim is directed to an immunogenic composition comprising a cholera holotoxin (five B subunits associated with the A subunit) and an antigen covalently associated with the CT, wherein the CT comprises an A subunit, which is mutated in position 29, and wherein CT increases the immunogenicity of the antigen.

   D1 discloses the linkage of the antigen -Saliva Binding Region of the streptococcal protein adhesin AgI/II- to Cholera Toxin A2/B, which is an ADP-ribosylating toxin in which a deletion of the fragment A1 (i.e. a mutation by deletion in the segment comprising residue 29) of the A subunit was carried out. D1 discloses that the entire antigen protein AgI/II induces S-IgA only when the antigen in conjugated to CTB, which implies that the protein alone does not induce S-IgA. The Saliva Binding Region, which is a fragment of the streptococcal adhesin AgI/II is immunogenic (see figure 5, b) when conjugated to Cholera Toxin A2/B and therefore the conjugation increases the immunogenicity of the antigen. Consequently D1 anticipates the subject-matter of claim 1. As Seq.Id.Nos 1 and 2 encode the entire subunit A and as claim 3 allows for any genetic modification including the amino acid in positions 29 and as claims 14, 15 comprise any

fragment of CT-A their respective subject-matter is not new (Article 33(2) PCT). Claim 2 specifies that the mutated CT has a reduced toxicity compared to the wild-type CT-A. As disclosed in D1 (abstract) that the toxic fragment A1 of the subunit A is deleted and consequently the toxicity is reduced in comparison to the wild-type CT-A. Therefore D1 anticipates also the subject-matter of claim 2 (Article 33(2) PCT).
D1 (figure 5) discloses also the combined use of the construct and an aluminium-based adjuvant and free cholera toxin (which is to be considered as a non-covalently attached antigen). Therefore D1 anticipates the subject-matter of claims 8-11 (Article 33(2) PCT). For the same reasons D1 anticipates also the subject-matter of independent claim 12 and dependent claims 13-15,29, 31, 32, 33,34.

2. The prior art does not disclose a construct with an additional antigen covalently attached to the CT, therefore the subject-matter of claims 7 and 30 is novel (Article 33(2) PCT). As the addition of a further antigen is a design option, which is not associated to any surprising effect, an inventive step cannot be attributed to the subject-matter of claims 7 and 30 (Article 33(3) PCT).

3. The subject-matter of claims 4,5, 16-28 is novel (Article 33(2) PCT) because the prior art does not disclose CT with one or more point mutations conjugated to an antigen.
D1 is considered as the closest prior art and the difference between the CT-antigen construct of D1 and of the present claims lies in the fact that in D1 the entire A1 fragment has been deleted in order to reduce the toxicity, whereas in the claims 4,5,16-28 single point mutations are carried out on the A1 fragment in order to reduce the toxicity.
The technical problem to be solved is the provision of an alternative modification of the A1 for reducing the toxicity of a CT-antigen construct. As already pointed out on page 11 of the description, the point mutations leading to a reduction of the toxicity are disclosed in the prior art (D3, D4, D5, D6) and the skilled person would solve the technical problem by combining the teaching of D1 and D2-D5 to arrive at the subject-matter of claims 4,5, 16-28 without involving any inventive effort. Therefore the subject-matter of claims 4,5, 16-28 does not involve an inventive step (Article 33(3) PCT).

4. As the method claims 52-85 are limited by the exact same features as the corresponding claims 1-34 to the compositions, the subject-matter of claims 52-54,57,59-62,63-67,80,82-85 is not novel (Article 33(2) PCT) for the same reasons as those given in the above section 1 for the corresponding claims to compositions. The subject-matter of the method of treatment claims 55,56,58,67-79 does not involve an inventive step (Article 33(3) PCT) for the same reasons as those given in the above sections 2 and 3 for the corresponding composition claims (Article 33(3) PCT).

5. For the assessment of the present claims 52-95 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

6. Documents D7 and D8 disclose also a construct in which an antigen is covalently linked to a CT-A2 subunit (i.e. a CT-A from which the toxic fragment A1 was deleted by mutation). Said documents are at least also novelty destroying for the subject-matter of claims 1, 52 and 12, 63 (Article 33(2) PCT).

Invention B: Claims 35-43, 86-94, which are directed to an antigen covalently associated to an Escherichia Coli heat labile toxin (LT).

7. Claim 35 is directed to an immunogenic composition comprising an Escherichia Coli heat-labile toxin (LT) and an antigen covalently associated with the LT, wherein the LT increases immunogenicity of the antigen. D2 (US6395964) discloses that antigens can be fused to LT or CT subunits and that the subunits are able to form holotoxins (col17, line 15-39). Thus D2 anticipates (Article 33(2) PCT) the concept of claims 35 and 86 that the antigen is fused to a non-mutated LT toxin.
D7 (see abstract) also discloses the subject-matter of claims 35 and 86, because LT comprising the non-toxic A2/B subunits is linked to a streptococcal antigen. D9 also anticipates the present claims 35 and 86, which are not limited to a holotoxin.

D9 discloses in claim 14 a fusion protein between a subunit B of LT and a viral antigen.

8. D10 (figures 1 A-C) discloses a Streptococcus mutans antigen fused to the A2 subunit of LT in combination with B subunits of LT. In D10 (page 2) the toxicity of the holotoxin was addressed by omitting the toxic A1 subunit and by providing an A2/B-antigen. The difference between the subject-matter of claims 2 and 87 lies in the fact that A subunits in the present claims are mutated and not omitted (as the A1 subunit in D10). The technical problem to be solved is the provision of an alternative non-toxic LT-antigen construct, which maintains an adjuvant effect on the immune response against the antigen. D11 and D3 disclose that the adjuvant properties of the LT-toxin are maintained and that the toxicity of the LT is abolished by mutating the serine in position 63 into a lysine or by mutating the alanine in position 72 into an arginine. In order to achieve the adjuvant effect without the toxic side-effects the skilled person would have combined the teachings of D10 and D11 or D3 with a reasonable expectation of success in order to arrive at the construct proposed by claims 36, 37 and 87, 88. Thus the subject-matter of the latter claims does not involve an inventive step (Article 33(3) PCT). It appears that the dependent claims 38-43 and 89-94 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, because said features merely refer to possible antigens and to well-known additional adjuvants.

Invention C: Claims 44-51, 95-101, which are directed to an antigen covalently associated with a pertussis toxin (PT).

9. D12 (see abstract) discloses a conjugate vaccine comprising a pneumococcal polysaccharide bound to a pertussis toxin and thus anticipates (Article 33(2) PCT) the subject-matter of claims 44 and 95, 96, 101.
D13 discloses a conjugate of an oligoside antigen and a pertussis toxin (claim 10; p. 10, l.9), which can be administered with further adjuvants (p. 12, l. 28). Therefore D13 anticipates the subject-matter of claims 44, 95, 96, 99, 101.
D14 discloses the use of a mutated (detoxified) pertussis toxin, conjugated to an HIV-antigen in a vaccine (see abstract). Therefore D14 anticipates the subject-matter of claims 44,45,46, 51,95, 96, 101 (Article 33(2) PCT). Dependent claims

47, 49, 50, 97, 98, 99, 100 appear to be novel but do not contain any features which, in combination with the features of any claim to which they refers, meet the requirements of the PCT in respect of inventive step (Article 33(3) PCT), because the addition of a further antigen or an adjuvant was not shown to have any technical effect.

Further Remarks:

10. Claims 59, 48, 40 are redundant (Article 6 PCT) because they are identical to their respective preceding claims.